Exhibit D

NOTEBOOK NO. 2610

ISSUED TO R. SAIKI

ON _____

DEPARTMENT 672

RETURNED _____ 19____

SCIENTIFIC NOTEBOOK CO.
5007 WEST DONNA DRIVE
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From Page No. X

The story going around is that NEB's Taq polymerase is losing activity in the hands of the users. We have solved that problem by adding non-ionic detergents to the storage buffer. Check the sample of Taq that NEB sent David (~20Mar87, see 2522:168) to see if it is still alive and if not, will 0.5% NP40/ 0.5% Tween 20 restore it.

A G M S:	10 u/100µl		
B H N T:	5	A-F:	"supernat" w/o detergent
C I O U:	2.5	G-L:	"supernat" with detergent
D J P V:	1.3	M-R:	"vortex" w/o detergent
E K Q W:	0.6	S-X:	"vortex" with detergent
F L R X:	0.3		

storage buffer: 100mM KCl, 10mM Tris 7.5, 5mM DTT, 0.1mM EDTA, 50% glycerol (NEB's ~~recipe~~ formulation)

Enzyme was not mixed, spun briefly in microfuge, and 5µl near meniscus diluted into 15µl storage buffer to get 20µl @ 5 u/µl (Cetus units, see 2522:170) - this is the "supernat" fraction w/o detergent. Two microliters (10u) was taken to prepare serial dilutions A-F as described below. To the remaining 18µl was added 1µl 10% NP40/10% Tween 20 (final: 0.5% each) to make the "supernat" with detergent fraction and 2µl taken for serial dilutions G-L.

The original tube of enzyme was vortexed to completely suspend any settled material. Enzyme was removed and distributed as above to prepare the "vortex" fractions and relevant serial dilutions.

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Witnessed & Understood by me.	Date	Invented by R. Saiki	Date
		recorded by	

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Molt4 @ 100 ng/ml, PC03 and PC04 @ 10 μM, dNTP @ 6 μM

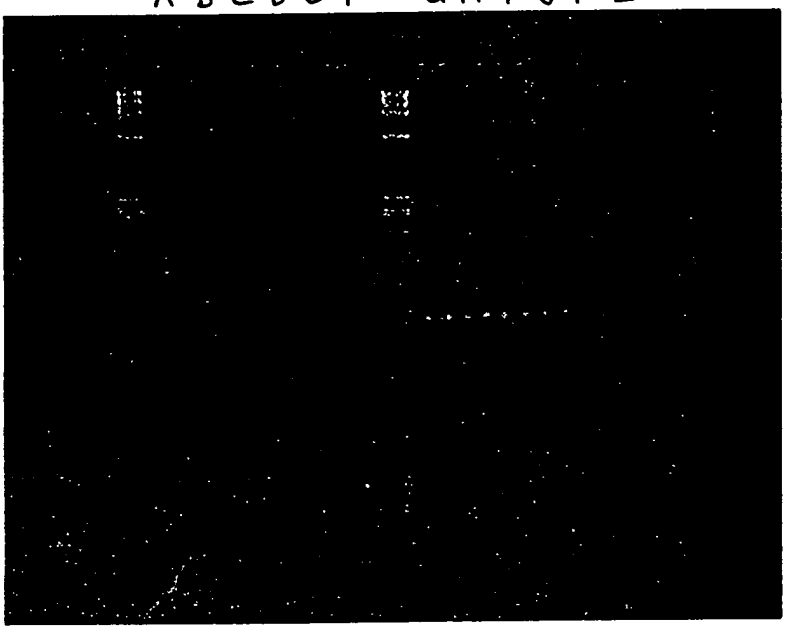
140 μl	Molt4	166 mM	$(NH_4)_2SO_4$
140 μl	10x NEB Taq Salts	670 mM	Tris 8.8
140 μl	PC03	67 mM	$MgCl_2$
140 μl	PC04	0.17%	gelatin
140 μl	dNTP		
700 μl	H_2O		
1400 μl	→ 4x 100 μl, 20x 50 μl		

Two μl (10 units) of the appropriately diluted enzyme (see previous page) was added to the 100 μl samples and five 50 μl two-fold serial dilution made for each. Samples were overlayed and subjected to 30 cycles using program described page 50 but with 0:30 min extension at 70°.

Load 5 μl each onto 3% NuSieve/1% agarose/1x TBE.

(−) (+)

A B C D E F G H I J K L



"supernat"

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Witnessed & Understood by me, [signature] Date

Invented by R. Saiki Date

Recorded by

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7

(–) (+)

MNOPQR STUVWX

"vortex"

Addition of detergents to storage buffer fully restores activity of enzyme. Compare to titration of lot U3-1 (page 61, A-F) to see that enzyme in (+) detergent fractions at 5 u/µl.

Without detergents there is no detectable amplification.

Don't see any significant differences between "supernat" and "vortex" samples. This suggests that the inactive enzyme does not readily fall out of solution.

Obviously, the aggregation problem NEB is having is readily solved by the addition of detergents to the storage buffer.

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Witnessed & Understood by me,	Date	Invented by R. Saiki	Date
Russell Higuchi		Recorded by	